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THE UNIVERSITY OF ALBERTA
REPRODUCTIVE BIOLOGY OF BROOK STICKLEBACK Culaea inconstans
IN ASTOTIN LAKE, ALBERTA

by



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A THESIS
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Reproductive Biology of Brook Stickleback Culaea inconstans in Astotin Lake, Alberta submitted by Brian David Smiley in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Aspects of reproduction in the brook stickleback Culaea inconstans (Kirtland) were studied in Astotin Lake, Elk Island Park during the summer of 1970. Quantitative field data on nesting and reproductive life history were compiled by underwater SCUBA observations, individual nest and fish collections and laboratory analyses of weekly fish samples.

The objective of the study was to describe the reproductive maturation and nesting behavior of Culaea under natural lake conditions.

The breeding period extended from mid-June to early August during which time the water temperature fluctuated between 16° and 26° C. A peak in nesting frequency (0.9 - 1.5 nests/m²) occurred on July 9. Gonad development in females was gradual during May. However an abrupt maturation trend in early June rendered up to 48% of the sampled population capable of breeding at the onset of spawning. The timing of the breeding cycle is examined in reference to existing literature on stickleback reproduction under laboratory and natural conditions.

The spherical or cigar shaped nest built by the male to house eggs and young is composed primarily of Rhizoclonium green algae and duckweed species strung around leaves and stem of its anchorage plant, Potamogeton spp. Data show that an aging nest becomes disorganized in form and larger in dimensions without the apparent addition of more building materials by the male. The general trend probably facilitates additional aeration of egg clutches by the guardian male and also provides a nursery or refuge for the newly hatched, yolked fry.

The distribution of nests throughout the rooted vegetation zone is restricted by such factors as water turbulence, plant distribution and

possibly light availability and predation. A direct positive correlation is shown between nest distance above the bottom and the water depth where water and wind conditions permit. However in regions exposed to wind and wave turbulence, the nests are built at substrate level regardless of water depth. All nests observed were in depths of 50 to 140 cm of water which corresponded to the extent of rooted aquatics in the littoral regions of the lake.

The clutches of nest eggs become depleted in size as embryo development proceeds from fertilization to hatching. Stomach analysis and field observations indicate that egg and fry mortality is due to combined factors of progeny cannibalism by the guardian male, predation by other sticklebacks and invertebrates, and the assumed natural causes of disease and infertility.

Comparison of parasitized and non-parasitized sticklebacks are made. Female Culaea infected with the plerocercoid larvae of Schistocephalus solidus (Müller, 1776) exhibit severe retardation in gonad size and oocyte development and are incapable of successful reproduction. The tapeworm larva comprises an average of 19.3% of the host fish body weight. Other pathology affecting both sexes are discussed.

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INTRODUCTION

In recent years, the sticklebacks have been widely utilized as "lab rats" in research of behavior and ethology (Lorenz, 1950; Tinbergen, 1952; Van Iersel, 1953; Morris, 1958), of experimental physiology (Swarup, 1959; Tromp-Blom, 1959; Hoar, 1962), and of aspects of evolution, speciation and geographic variation (Lindsey, 1962; Hagen, 1967; McPhail, 1969; Nelson, 1969; Moodie, 1970). The over-all suitability of the stickleback for such specialized study has resulted in general neglect of background life history studies and reproductive biology of the Gasterosteidae, particularly of the brook stickleback Culaea inconstans (Kirtland). The bulk of present evidence of Culaea biology is founded predominantly on observations under artificial closed conditions of aquaria. Barker (1918), Jacobs (1948), Winn (1960) and Reisman et al (1967) are some authors that have published more than a note on field observations of the breeding habits of Culaea. The purpose of this study is to present primarily field and underwater observations of Culaea reproduction, quantified where possible with environmental data and laboratory analysis of sampled fish. Aspects of sexual maturation during the nesting cycle are emphasized.

Due to the high incidence of infection of fish with Schistocephalus solidus plerocercoid in Astotin Lake, further consideration is given to the pathological effects of infections on successful reproduction of Culaea. The pathology of parasitization by pseudophyllidae larvae are well documented and have been reviewed by Williams (1967) and Arme and Walkey (1970). Typical symptoms are interferences with host growth rate, maturation processes and swimming activity.

DESCRIPTION OF AREA AND HABITATS STUDIED

Astotin Lake (Lat. $53^{\circ}41'N$, Long. $112^{\circ}51'W$) is the largest of several lakes located within Elk Island National Park, Alberta. The lake, situated 37 km (23 mi) east of Edmonton, lies in knob and kettle moraine formed by the retreat of the Wisconsin glacier from Central Alberta. The triangular-shaped lake has an area of 5.616 km^2 and a mean depth of 3.04 m (maximum depth of 7.01 m). Because of its shallowness and limited water exchange, Astotin Lake is broadly classified as an eutrophic body of water characterized by high summer temperatures, rich nutrient resources and seasonal periods of low oxygen. The lake bottom, consisting of organic ooze deposits of autochthonous origin, slopes gradually from the shoreline. In shallow regions of the lake the substrate is covered by a mat of benthic and epiphytic filamentous algae, predominantly Rhizoclonium sp. Sand and gravel bottoms occur in windswept exposures of the lake.

Astotin Lake has an abundant invertebrate fauna (Lin, 1968) which in turn supports large numbers of breeding and migrant waterfowl (Kevan, 1970) as well as one species of teleost fish, the brook stickleback. Prior to 1932, fishes present in the lake included northern pike, Esox lucius, yellow perch, Perca flavescens, and brook stickleback. A dam erected on Martin Creek (Astotin Creek), the outlet stream draining into the North Saskatchewan River, now prevents seasonal migration of spawning fish and inhibits normal lake drainage (Lin, 1968). The resulting accelerated eutrophication and susceptibility to winter and summer kills eliminates all fish species except Culaea which can thrive under conditions of low oxygen tension.

Three study areas and collection sites representative of the morphometry, fauna and flora composition of Astotin Lake were chosen (Fig. 1). From 1969 summer observations, these sites were known to be utilized as spawning environments by Culaea and were easily accessible by road and/or boat.

RESIDENCE POINT is a sheltered shallow bay with good wind and wave protection afforded by the proximate treed shoreline. The bottom is soft mud and organic debris with a few scattered areas of sand. An active beaver lodge is located near the collection site. A 30 m belt of rooted macrophytes grows parallel to the shoreline and extends to a depth of 1.7 m. Potamogeton richardsonii, the dominant macrophyte, is randomly distributed throughout the region and reaches particularly dense numbers during July and August. Small isolated clumps of P. filiformis are found in 0.5 to 1.0 m of water while Myriophyllum verticellatum and Ceratophyllum demersum grow in low frequency along the furthest extension (depth of 1.5 to 1.7 m) of the rooted aquatic belt.

HIGH ISLAND is located near the centre of the lake in open water and consequently is exposed to frequent northwest winds and heavy wave buffeting. The mud-sand substrate slopes moderately to approximately 7 m, the maximum depth of the lake. The vegetation zone is limited to a narrow belt, 15 m wide, of predominantly P. richardsonii, reaching a depth of 1.3 m. Occasional patches of P. natans and P. filiformis are growing throughout the area.

YOUTH HOSTEL BAY, the third site studied, experiences similar wind and wave conditions to those of High Island. The bottom is composed of sand, large pebbles and mud. Potamogeton species are represented

Fig. 1. Astotin Lake, Elk Island National Park, Alberta. The 3 study areas are indicated by black arrows and the line-transects are designated by stipled lines at Residence Point and High Island.

Martin Creek



N

Residence Point



High Island



Youth Hostel Bay



Inlet Stream

1 km



1 mile



in low numbers, with P. richardsonii as the dominant species.

Frequently the water becomes saturated with accumulations of blue-green algae Aphanizomenon sp and Anabaena sp following storms or windy periods.

MATERIALS AND METHODS

A. General Organization

A preliminary survey of the lake was made in the summer (June to August) of 1969. The bulk of data was collected in the period April 27 to October 15, 1970. Semi-weekly trips to the lake were made to sample fish, record chemical and physical data and obtain nesting data on Culaea. Underwater observation and collection required 90 logged hours of SCUBA and snorkel diving.

B. Collection of Fish Samples

Twenty-one weekly collections of sticklebacks were netted from three study areas. A 3/16" ace-oval mesh seine net (6.1 m X 1.2 m) (20' X 4') was used to collect each sample consisting of approximately 500 fish of all size classes. The fish were fixed immediately in 10% formalin, labelled and later preserved in 40% isopropyl alcohol.

C. Collection and Analysis of Breeding Males and Nests

Breeding males were individually captured while at their nests or while escorting a swarm of fry. SCUBA equipment and a "slurp gun", a suction device with a one-way trap door, were employed to capture male Culaea. Following this operation, each associated nest structure was measured for distance above the lake substrate, using a 5 m plastic tape measure attached to a styrofoam float. The nest and a piece of its supporting vegetation were collected, preserved in 10% formalin and labelled. All data including nest height, water depth, substrate types and behavioral observations were immediately dictated into a portable tape recorder and later transcribed into field notes.

In the laboratory the diameters of 146 nests were measured with dial-calipers across the widest point of the structure. Each nest was teased apart and the clumps of eggs, if present, were removed and counted. The nests were categorized on the basis of the developmental stage of these egg clutches as they appeared under a low power microscope. "Uneyed eggs" were recently fertilized zygotes with no evidence of body structures. Eggs with apparent eyes in the shape of half-moons and a curved vertebral column were arbitrarily called "1/2 eyed eggs". When the developing egg contained an embryo with two obvious eyes, a definite body and tail and a large yolk sac, it was categorized as an "eyed egg". All nest materials except the anchorage plant were blotted dry on paper towelling and immediately immersed in a 10 ml graduated cylinder of water. The displaced volume of each nest was recorded. Construction materials used in building were then identified.

The guardian male collected along with each nest was measured for standard length and then analysed for stomach contents (presence or absence and numbers of ingested stickleback eggs and fry). The general stage of development of any ingested eggs was determined with the same criteria used for the nest clutch eggs.

D. Transect Data Collection

On July 7, two transect lines were chosen adjacent to collection sites at High Island and Residence Point (Fig. 1). The locations, representing the flora and substrate types, were known sites of high nesting activities. High Island line-transect was 12 m (39') in length (divided in 1/2 m units) and extended normal (at right angles) from the shore to the outer edge of the rooted aquatic zone (depth of 114 cm).

Similarly a 25 m (82') line-transect was required at Residence Point to span the potential spawning environment to a maximum depth of 130 cm.

Each transect was "run" once on July 7, 9, 11, 15, 22, 29 and August 5 using SCUBA techniques for nest observation. Within a distance of 1 m on either side of the transect, every nest observed was measured for vertical distribution (distance above substrate measured to the centre of the nest) and horizontal distribution (distance normal to the shore). In addition, absence or presence of guardian males, egg clutches or fry were noted. The total nesting areas covered by the transect study was 24 m^2 (258 ft^2) at High Island and 50 m^2 (538 ft^2) at Residence Point.

E. Gonad and Host Parasite Analyses

Thirty-three collections of 30 randomly-chosen female sticklebacks, free from Schistocephalus solidus infections, were analysed for development of ovaries and fecundity (numbers of ripe ova of 1.1 - 1.2 mm diameter in both ovaries). The total 990 fish represented the three study areas and eleven collection dates between April 30 and August 4. The sampled females were measured from snout to the end of the fleshy caudal peduncle. After removal of excess water with paper tissue, each fish was weighed on a Mettler pan balance. Both ovaries were removed and immediately weighed in glycerin to prevent weight loss due to water evaporation. The ovaries were then teased apart in water and the ova separated. The mean diameter of the largest oocytes was measured with dial-calipers. If the diameter exceeded 0.6 mm, the oocytes were counted with the aid of a hand-tally.

To provide a measure of the gonad weight, relative to the weight of individual fish, a gonadosomatic index (GS. I.) was calculated according

to the formula:

$$\frac{\text{Total weight of both ovaries}}{\text{Total weight of fish plus ovaries}} \times 100$$

A GS. I. of 10 thus refers to a female whose ovaries account for 10% of the total body weight.

Fifty females and 10 males infected with the plerocercoid larvae of the pseudophyllidean cestode Schistocephalus solidus were randomly selected from June 9 collections from the three study areas. In addition 50 females and 10 males, non-infected, of similar size were randomly taken from the same samples to represent the normal population and to serve as a comparison for seasonal gonad development. After measuring the standard length with dial-calipers, each fish was weighed on a Mettler pan balance. Both ovaries were removed and immediately weighed in glycerin. In the case of a host fish, the plerocercoid worm(s) was also weighed in glycerin. Gonadosomatic indices (GS. I.) were calculated for all fish, as well as the parasite load (%) of host fish according to the formula:

$$\frac{\text{Weight of the plerocercoid(s)}}{\text{Weight of the host fish plus parasite}} \times 100$$

LIMNOLOGY AND WATER CHEMISTRY

From May 7 to August 18, physical and chemical data were recorded weekly with additional semi-weekly readings of the lake water temperature. The measured readings of pH, alkalinity and oxygen concentrations are shown in Fig. 2. These data are averaged values recorded at the 3 study sites.

The pH, recorded with a Metrohm #E2804, fluctuated irregularly throughout the study period, ranging in value from 8.7 to 9.6.

Alkalinity tended to decrease during the summer although abrupt fluctuations of 1 - 2 grains/gal (17 - 34 ppm) occurred at irregular intervals. Total and calcium hardness did not vary from 7 grains/gal (120 ppm) and 3 grains/gal (51 ppm) respectively. A Hach Kit PO-9A was used for these tests.

Oxygen concentrations were determined with a dissolved oxygen Hach Kit #OX-10 using the azide modification of the Winkler method of determination. Oxygen values ranged from 5.5 to 12.0 ppm but a concentration of about 8 ppm was usual throughout the breeding period. Apparently the abnormally windy spring and summer prevented oxygen levels from approaching expected low values of less than 3 ppm (Lin, 1968).

The water temperature, as recorded on Werbe maximum-minimum thermometers and a Tele-thermometer #43TD, increased during May, June and early July with occasional very warm or exceptionally cool periods (Fig. 3). After reaching a peak maximum of 26°C on July 14, the lake began to cool slowly until the end of August. About one week prior to commencement of spawning, warm calm weather caused an

Fig. 2. The pH, alkalinity (total) in grains/gal, and oxygen in ppm recorded during the period May 5 - August 14, 1970.

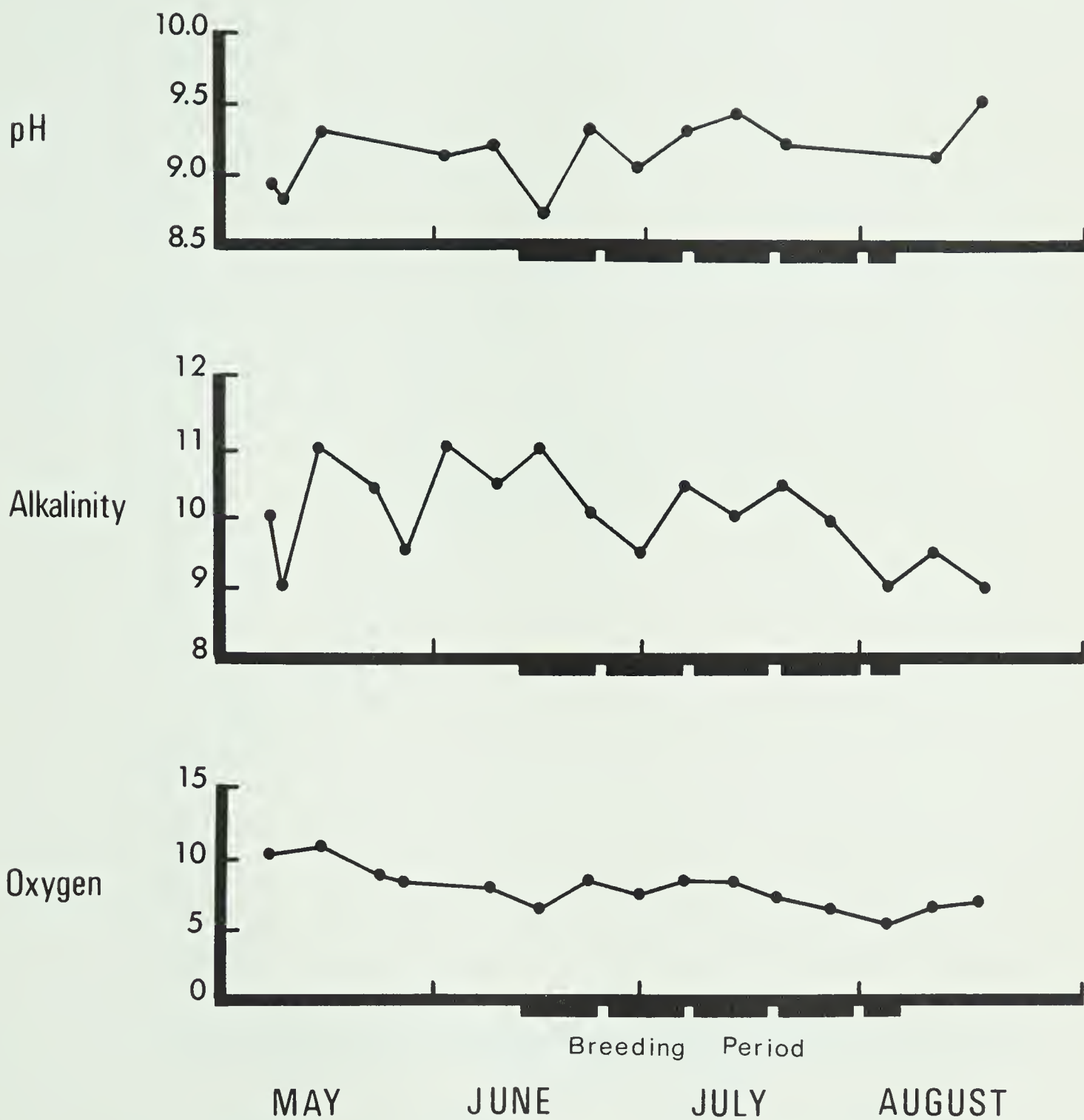
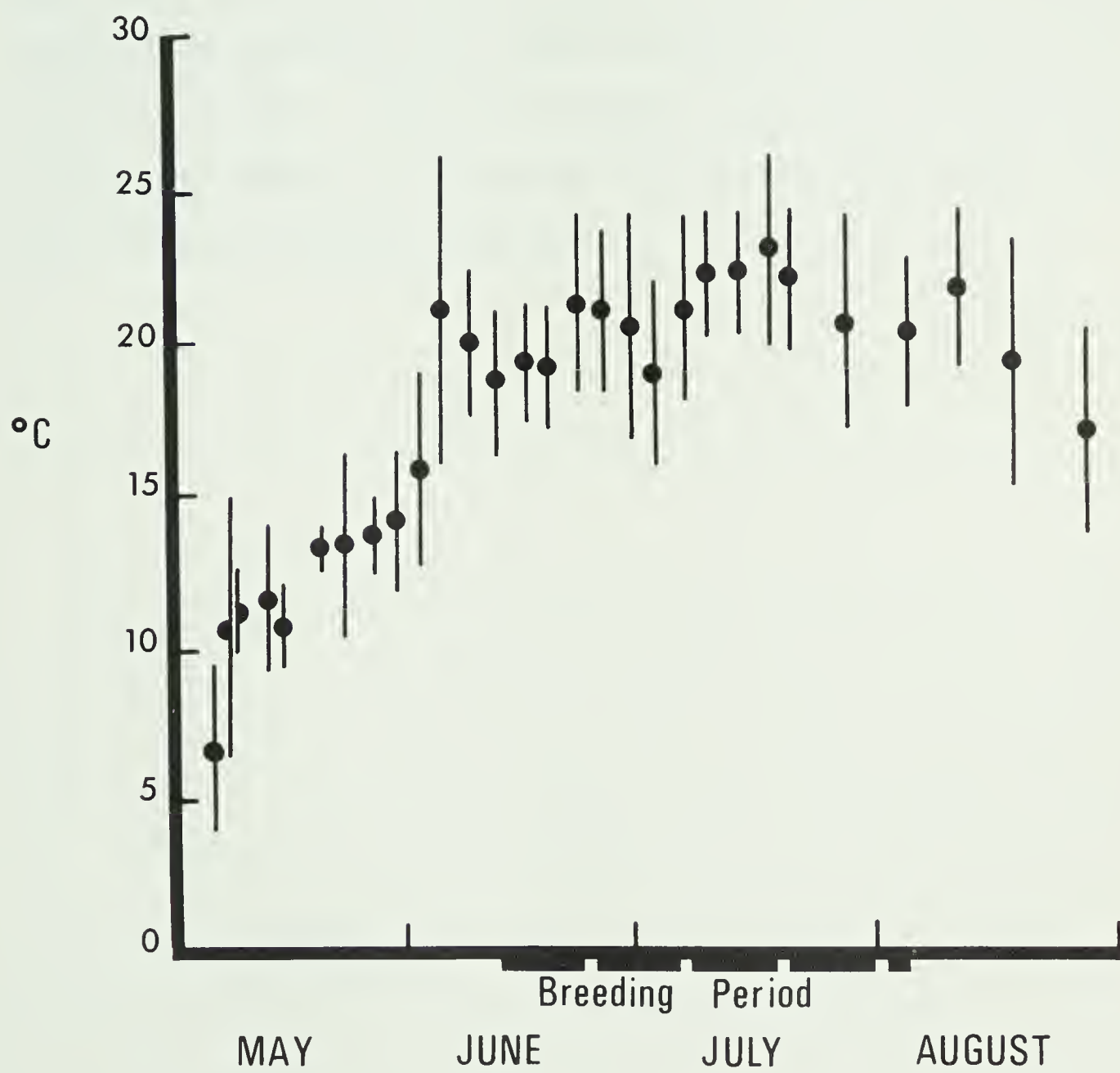


Fig. 3. The maximum-minimum temperatures ($^{\circ}\text{C}$) recorded in Astotin Lake, 1970. The represented values are the averages measured at the 3 study site locations. The black dots show the mid-point of each temperature range.



abrupt increase in lake temperature, which reached a maximum of 26°C . Prior to June 5, I did not record temperatures above 18°C . During the breeding period, the mean temperature was about 21°C although considerable ranges of values were recorded ($16.0 - 26.0^{\circ}\text{C}$).

Temperature readings, taken along the shoreline in 5 to 10 cm of water and at the surface and substrate level in 1 m of water, revealed that the lake temperature was almost homogenous throughout the reproductive period. Within the different strata of the vegetation-nesting environment, the temperature did not deviate more than 3°C ($\bar{x} = 0.9^{\circ}\text{C}$) on any particular date.

REPRODUCTIVE BIOLOGY OF Culaea inconstans

A. Breeding Period and Seasonal Maturation

1. Duration of breeding cycle

In Astotin Lake, underwater observation on June 12 revealed 23 and 10 nests of Culaea at Residence Point and High Island study areas respectively. Male sticklebacks in dark nuptial colors were actively defending nests and territories while aggregates of 15 to 20 gravid females were present throughout the nesting area, apparently searching for food items. For two weeks prior to June 12, male fish were engaged in intra-specific threats and fights or territoriality, but no cases of nest-building were recorded. Nesting activity persisted until July 29 at which time only one active nest (protected by a male) was noticed during four hours of diving. No nests were located after this date.

2. Nesting frequency

Although nesting frequency was not quantified throughout the entire spawning period, the line-transect data (Fig. 4) show a peak in nesting activity on July 9. Thirty-seven nests (1.5 nests/m^2) at High Island and 46 nests (0.9 nests/m^2) at Residence Point were recorded on this date. Nest frequency declined rapidly until the end of July and completion of spawning. A total of 247 different nests were located from both transects during the period of July 7 to August 5.

3. Gonad development of females

The data shown in Fig. 5 represent the seasonal maturation of the ovaries of female Culaea from pre- through post-spawning periods. The mean percentage weight of the ovaries of females increased gradually from April 30 to May 26 with GS. I. values ranging close to

Fig. 4. The numbers of stickleback nests located during underwater observation along the line-transects at Residence Point and High Island.

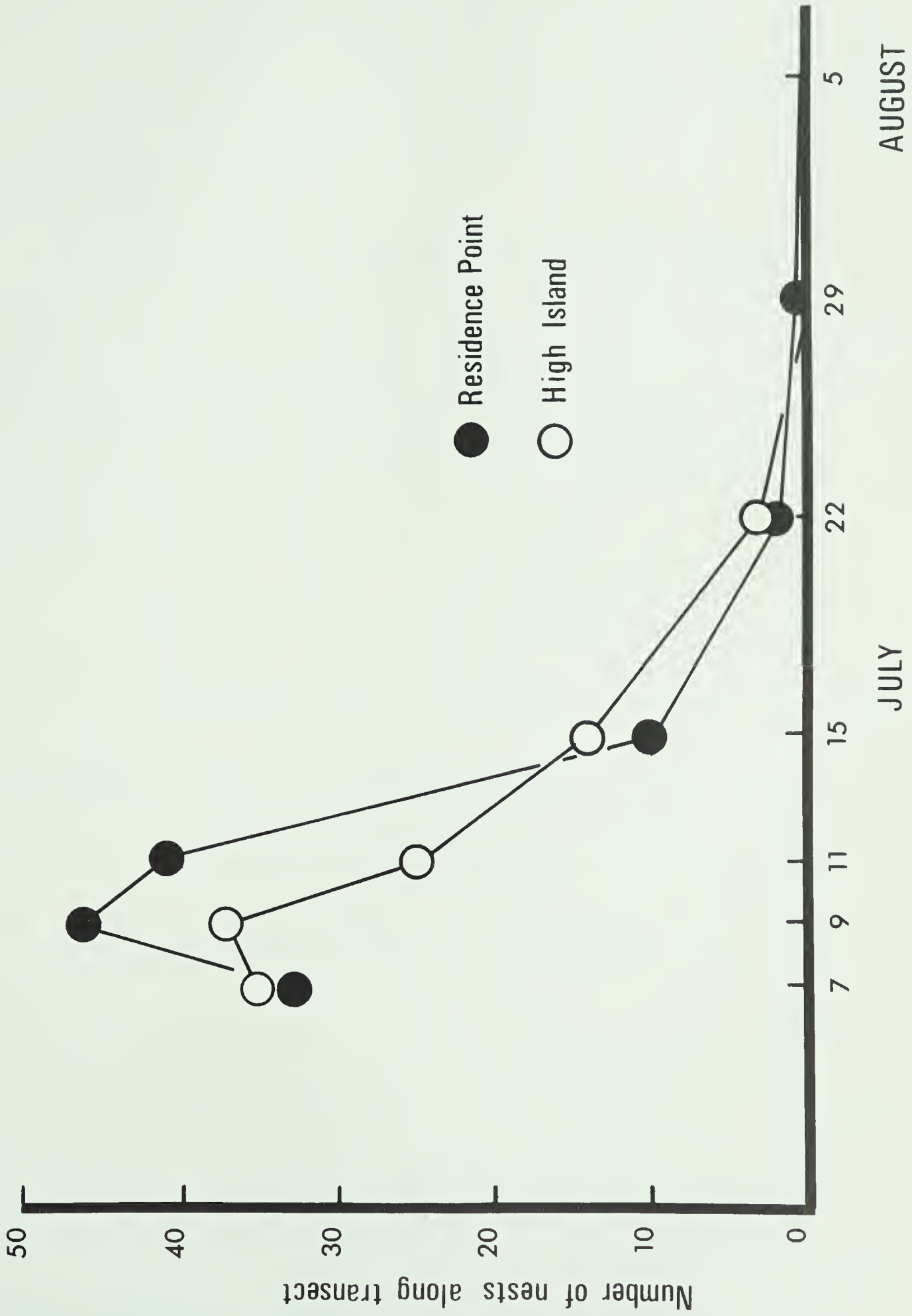
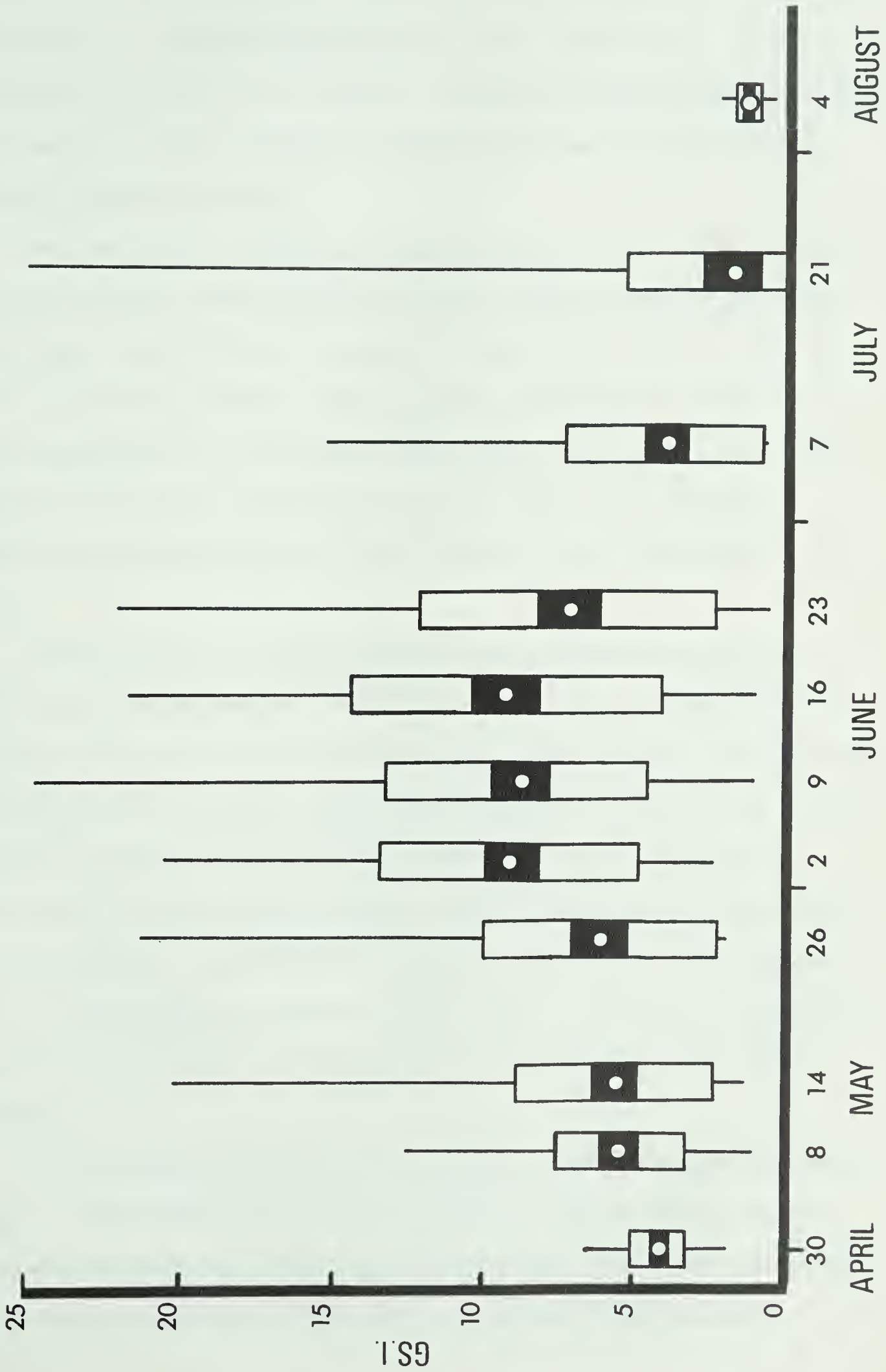


Fig. 5. Data illustrating the seasonal development of ovaries (represented as gonadosomatic indices, GS. I.) in female Culaea. Black bar, 2 standard errors of the mean; black bar plus white bar on either side of the mean, 1 standard deviation; vertical line, range.



5.0 - 6.0. After a rapid increase for one week (May 26 - June 2), the mean GS. I. remained approximately 9 until mid-June, reaching a maximum value of 9.2 on June 16. A decrease followed and continued until August 4. No spawning or nesting activities were observed in Astotin Lake after July 29.

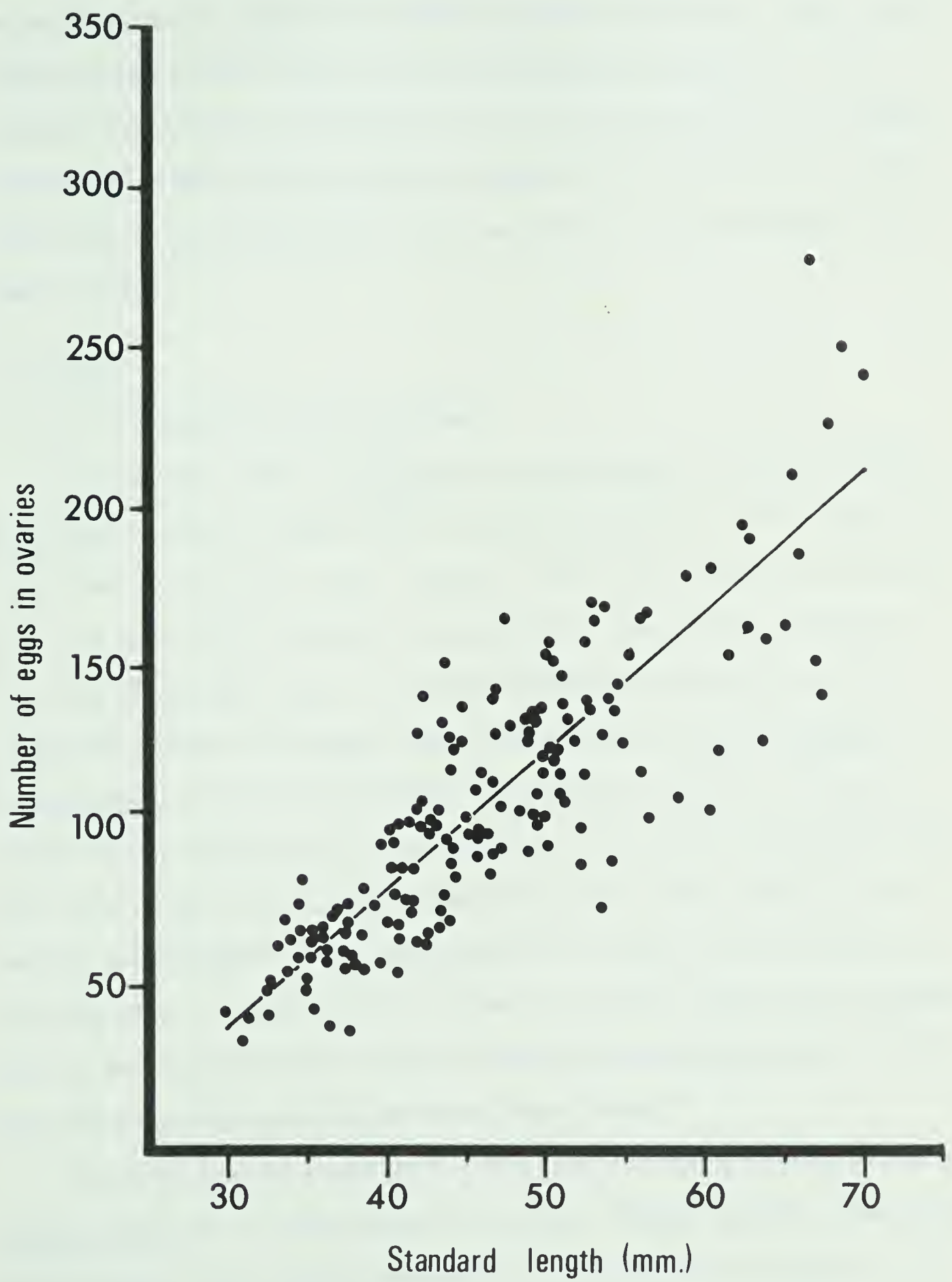
The mean GS. I. values are depressed in value and therefore relative because of the inclusion of ovary weights taken from female individuals who likely were not approaching sexual maturity for the 1970 reproductive period. The immature and "spent" females were indistinguishable by microscope observation since oogonia and small oocytes (less than 0.5 mm) were present under both conditions. Histological preparations of ovarian tissue was not undertaken in this study.

Before May 14, no sampled female was capable of immediate spawning since the oocytes within the ovaries were smaller than 1.1 - 1.2 mm, the normal size of mature eggs. Even on May 14 and 26, only 3% and 16% of the females respectively had fully developed ova. The maximum number of reproductively mature females (48%) were measured from the June 16 sample. For two weeks preceding June 16, the percentage of ripe females remained close to 39%. The decline in GS. I. and frequency of mature females after June 16 was a function of the onset of spawning and the appearance of "spent" females in the samples.

The relationship between egg production and fish length is shown in Fig. 6. One hundred and eighty specimens collected before June 12 (initiation of spawning) were examined for these data. The calculation of the linear regression of fecundity on standard length shows a

Fig. 6. Egg production (fecundity) of female Culaea collected before June 12, the start of spawning and nesting.

($n = 180$, $Y = -95.6 + 4.4X$)



significant, direct correlation ($r = 0.606$, $p < 0.01$, $df = 150$). Although at any particular length the fecundity extends over a wide range, mean values of egg counts tend to rise as the fish size increases. The maximum fecundity recorded was for a 66.7 mm female with 279 eggs. Egg retention after the spawning act appears to be minimal since only three fish were recorded with 1 to 5 ova of 1.1 - 1.2 mm diameter in their ovaries.

B. Nests

1. Description and construction

The nesting edifice of Culaea is an assemblage of leaves, sticks and algae varying in shape from a compact, spherical ball or egg-shape to a spinning-top form (Figs. 7 and 8). The nest entrance, an obvious hole of 1.0 - 1.5 cm diameter is located near the centre or bottom of one side of the nest (Fig. 7). In the majority of nests, the male stickleback glues the cottony, wind-blown seeds of aspen (Populus tremuloides) around the circumference of the hole, giving a "down-lining" appearance to the entrance. The fish likely obtains this nest item on the surface of the water where the fluffy seeds fall and become waterlogged. The fruiting period of aspen occurs during July. Occasionally, a similar-sized, inconspicuous hole opens on the opposite side of well-defined nests, likely created by spawning females during exit after egg deposit (also noted by Winn, 1960).

The predominant architectural component of the stickleback nest is Rhizoclonium sp, a green filamentous alga (Figs. 8c, 8d). This wiry, wooly alga covers large areas of lake substrate with a dense and tangled blanket. Because of its coarse texture and availability, Rhizoclonium is the preferred and almost exclusive nesting material.

Algae Cladophora sp and Ulothrix sp, additional Myxophyceans present in Astotin Lake, are rarely incorporated into the nests. Males are often observed carrying pieces of Lemna trisulca duckweed in their mouths to be used in nest construction. The entire bundle of algae and other vegetation is bound tightly together with a thread-like, mucous secretion manufactured in the kidneys of the male fish (Barker, 1918; Jacobs, 1948; Winn, 1960; Wai and Hoar, 1963).

The basic framework of the nest is fabricated around the rooted Potamogeton plant to which it is anchored. The crinkly leaves and stiff stems of P. richardsonii or the long, thin leaves of P. filiformis are employed as scaffolding to which Rhizoclonium strands are molded into place by the male (Figs. 7a, 7c, 8a, 8b).

2. Support preferences

Potamogeton spp are used almost exclusively for nest anchorage. Other rooted macrophytes such as Ceratophyllum sp and Myriophyllum exalbescens are not utilized for nest anchorage. Of the 159 nests collected, eighty-seven (55%) were anchored to P. richardsonii. Either one, two or three individual plants (in 56, 28 and 3 nests respectively) were manipulated into the nest framework. An arrangement using a two stemmed support is shown in Figure 7d. In 12 cases, the nests were organized within the branching, tangled network of the root system (Fig. 7c). Using his mouth and tail, the male exposes the roots by removal of all debris, algae and loose sediment within a radius of approximately 15 cm. Consequently, the resulting nest is level with or slightly below the normal substrate level. Excavation of any algae or sediment down to a sand-gravel substrate was

Fig. 7a. Newly constructed nest anchored to the stem and leaves of Potamogeton richardsonii.

Fig. 7b. Compact, tightly-bound nest showing evidence of the silky, thread-like secretion produced by the male to secure the algae into a dense structure.

Fig. 7c. A somewhat degenerated nest constructed within the root network of P. filiformis.

Fig. 7d. Stickleback nest constructed between two individual plant stems of P. richardsonii and incorporating the leaves into the framework.



Fig. 7a



Fig. 7b



Fig. 7c

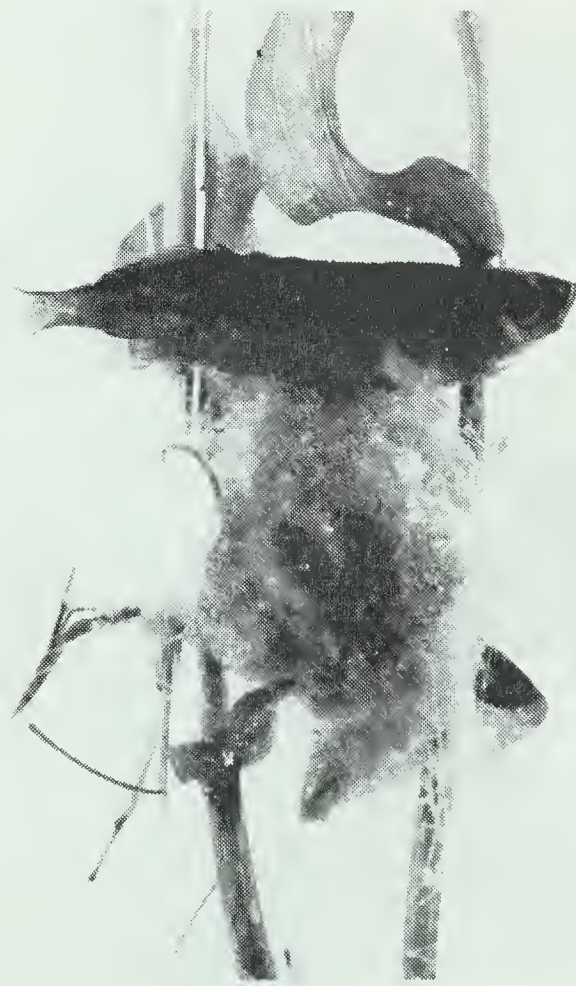


Fig. 7d

Fig. 8a. A stickleback nest shaped like an inverted spinning-top.
The nest contained "eyed" eggs.

Fig. 8b. Disorganized nest containing "eyed" eggs. Fibres of
P. filiformis in addition to Rhizoclonium were used to
construct the nest on P. richardsonii stem.

Fig. 8c and 8d. The completely-undefined tattered remains of
nests which provide continued protection as a "nursery"
for the yolked fry.



Fig. 8a



Fig. 8b



Fig. 8c



Fig. 8d

undertaken by males whose nests were 10 cm or less above the bottom.

Nine nests were fastened to submergent deadfall of Populus tremuloides; seven had no support at all but were located within small depressions excavated in the blanket of Rhizoclonium. One nest was erected inside a Libby's Tomatoes can.

3. Nest size

The stickleback nest undergoes an obvious increase in diameter as the egg development progresses (Fig. 9). Newly constructed, compact nests without egg clutches had a mean diameter of 19.0mm. Once the clutches had reached the eyed egg or fry stage, the tattered remains of nests had increased to a diameter of about 33mm. An unpaired t-test shows that the increases in diameter are significant between most successive categories of clutch development (legend of Fig. 9). The only exception is the increment between categories 3 and 4. An insignificant decrease ($p > 0.05$) in diameter occurs between the time of hatching and the eventual abandonment of the nest by the male and fry (categories 5 and 6), probably due to the loss of nest materials from the disintegration of the nest structure.

The nest volumes exhibit extreme variation in values (0.20 - 1.40 ml) within any one category based on egg ontogeny (Fig. 10). The mean displaced volumes of nesting materials show no significant correlate with the stages of breeding cycle. The average nest volume was about 0.50 - 0.70 ml. The one exception to the lack of correlation is a significant increase between categories 2 and 3 (legend of Fig. 10).

The size (volume) of stickleback nests is no greater for large males than for small males ($r = 0.115$, $p > 0.05$, $df = 125$).

Fig. 9. Data illustrating changes in nest diameter as egg clutches develop from recently fertilized eggs to yolked fry. The sample size of nests examined (N) is included in the figure. Below are listed statistics of unpaired t-test analysis between categories:

1 and 2	$t = 6.221, p < 0.001, df = 90$
2 and 3	$t = 2.830, p < 0.010, df = 80$
3 and 4	$t = 1.892, p > 0.050, df = 24$
4 and 5	$t = 2.466, p < 0.025, df = 25$
5 and 6	$t = 1.692, p > 0.050, df = 29$

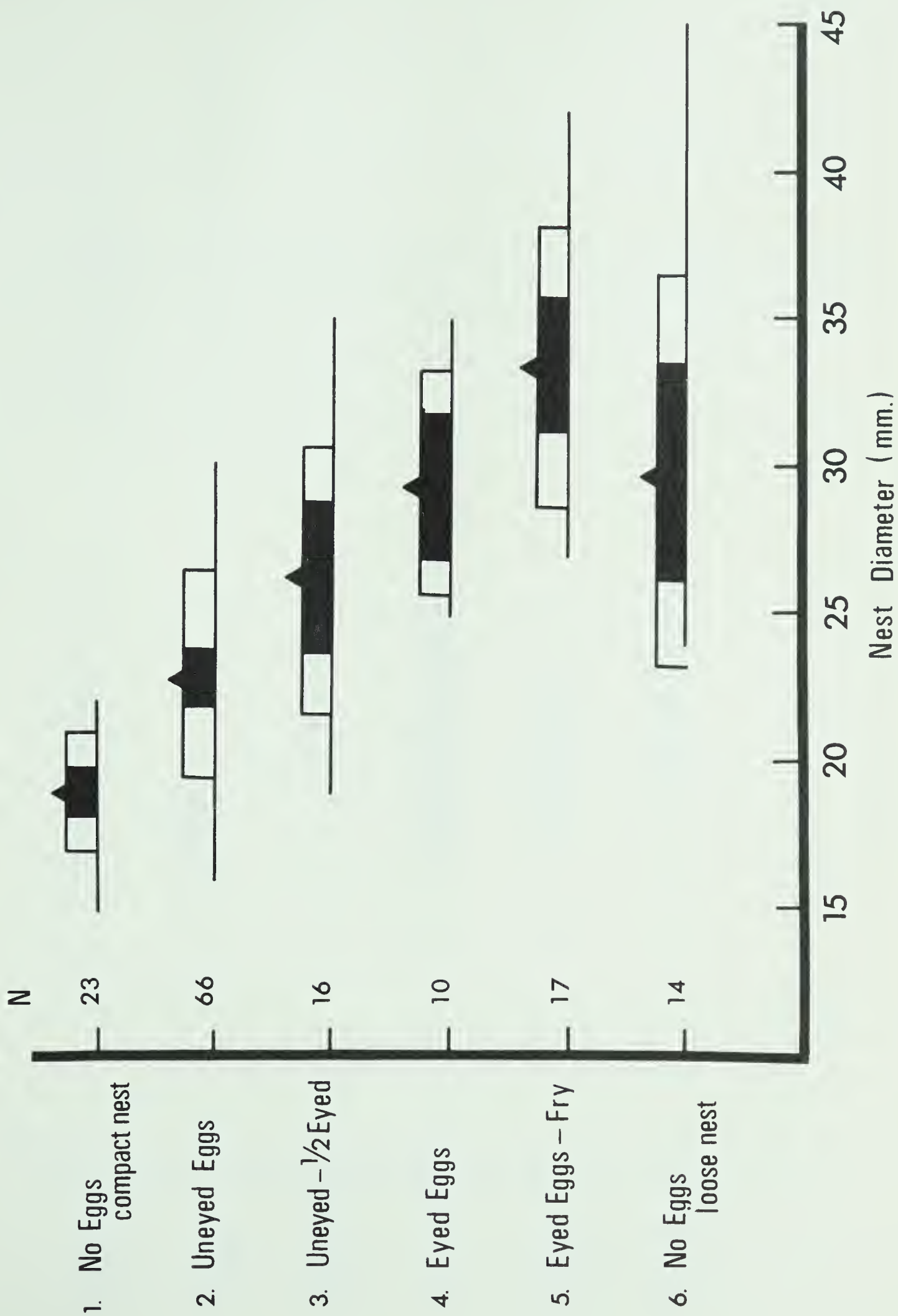
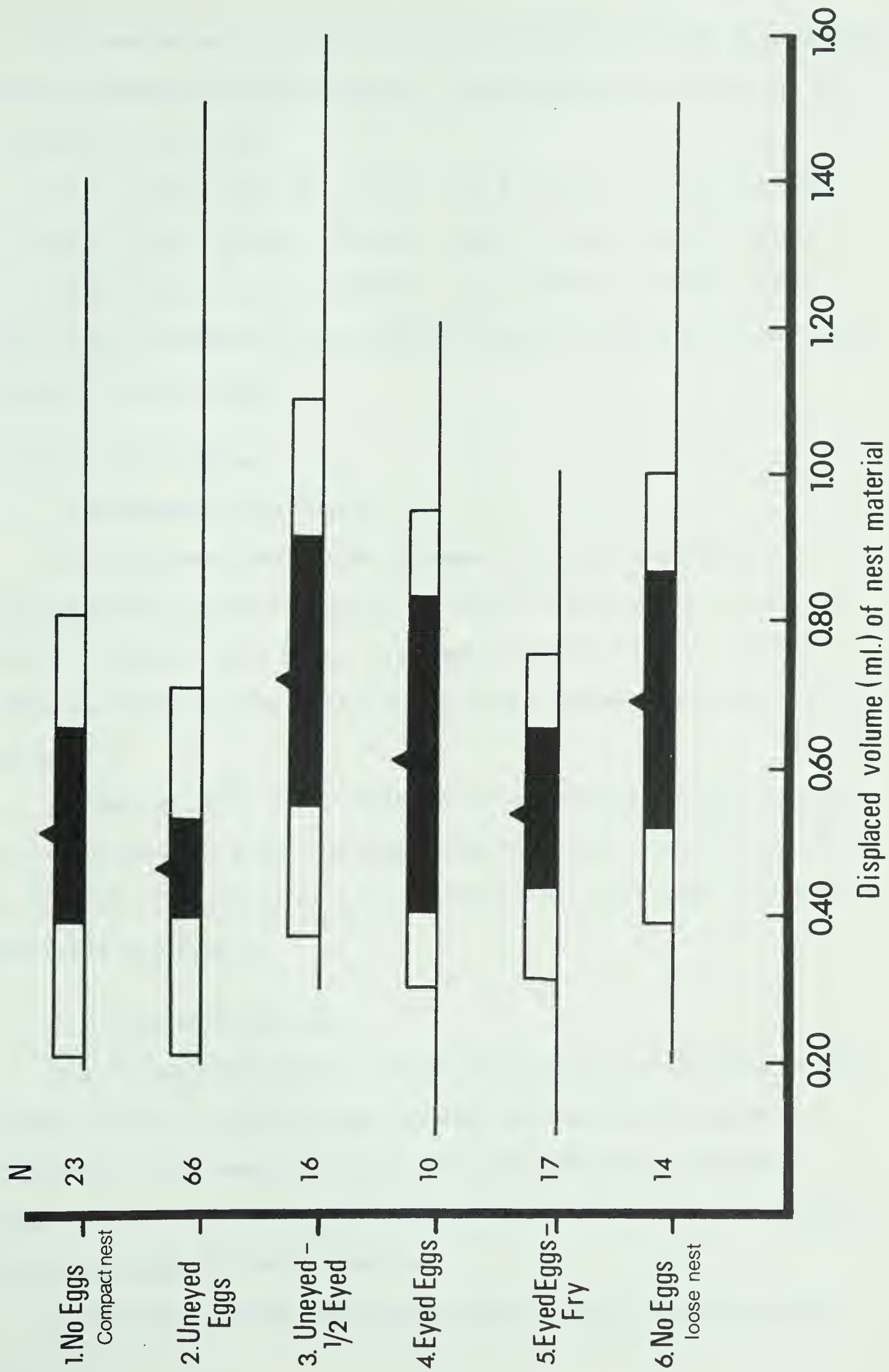


Fig. 10. Displaced volumes of nesting material used in construction of the nest. Each nest was categorized on the basis of general stage of embryonic development of eggs contained within the nest. The sample size (N) is shown. Below are listed statistics of unpaired t-test analysis between categories:

1 and 2	t = 0.651,	p>0.500,	df = 90
2 and 3	t = 2.816,	p<0.010,	df = 80
3 and 4	t = 0.789,	p>0.400,	df = 24
4 and 5	t = 0.653,	p>0.500,	df = 25
5 and 6	t = 1.522,	p>0.100,	df = 30



No consistent, significant correlation is found between nest volume and nest diameter in each category. The correlation coefficients for categories 1 to 6 are:

- (1) $r = 0.234$, $p > 0.05$, $df = 21$; (2) $r = 0.472$, $p < 0.01$, $df = 64$;
 (3) $r = 0.315$, $p > 0.05$, $df = 14$; (4) $r = 0.446$, $p > 0.05$, $df = 8$;
 (5) $r = 0.026$, $p > 0.05$, $df = 15$; (6) $r = 0.667$, $p < 0.05$, $df = 12$.

Thus nests increase in outside diameter with the addition of little or no construction materials.

C. Nest Distribution

1. Horizontal distribution

The horizontal distribution (distance normal from shore) of Culaea nests measured along the transects is represented in Fig. 11a and 11b. At Residence Point, nests were scattered from 3 to 25 m offshore. However the highest proportion of nests were found at 20 to 22 m.

In contrast, High Island nests were constructed in high numbers close to shore (4 - 7 m) reaching a peak frequency of eighteen at the 4 - 5 and 6 - 7 m points along the transect. No nests were observed beyond 16 m offshore.

2. Vertical distribution

The vertical distribution (height of nest above the bottom) is given in Fig. 12 and 13. These measurements were derived from two independent data sources — individual nest collection and transect observations. For ease of comparison these data are represented on separate graphs for each study area.

At Residence Point, the transect data indicate that the height at

Fig. 11a. Histograms showing nesting frequency along the RESIDENCE POINT transect.

Fig. 11b. Histograms showing nesting frequency along the HIGH ISLAND transect.

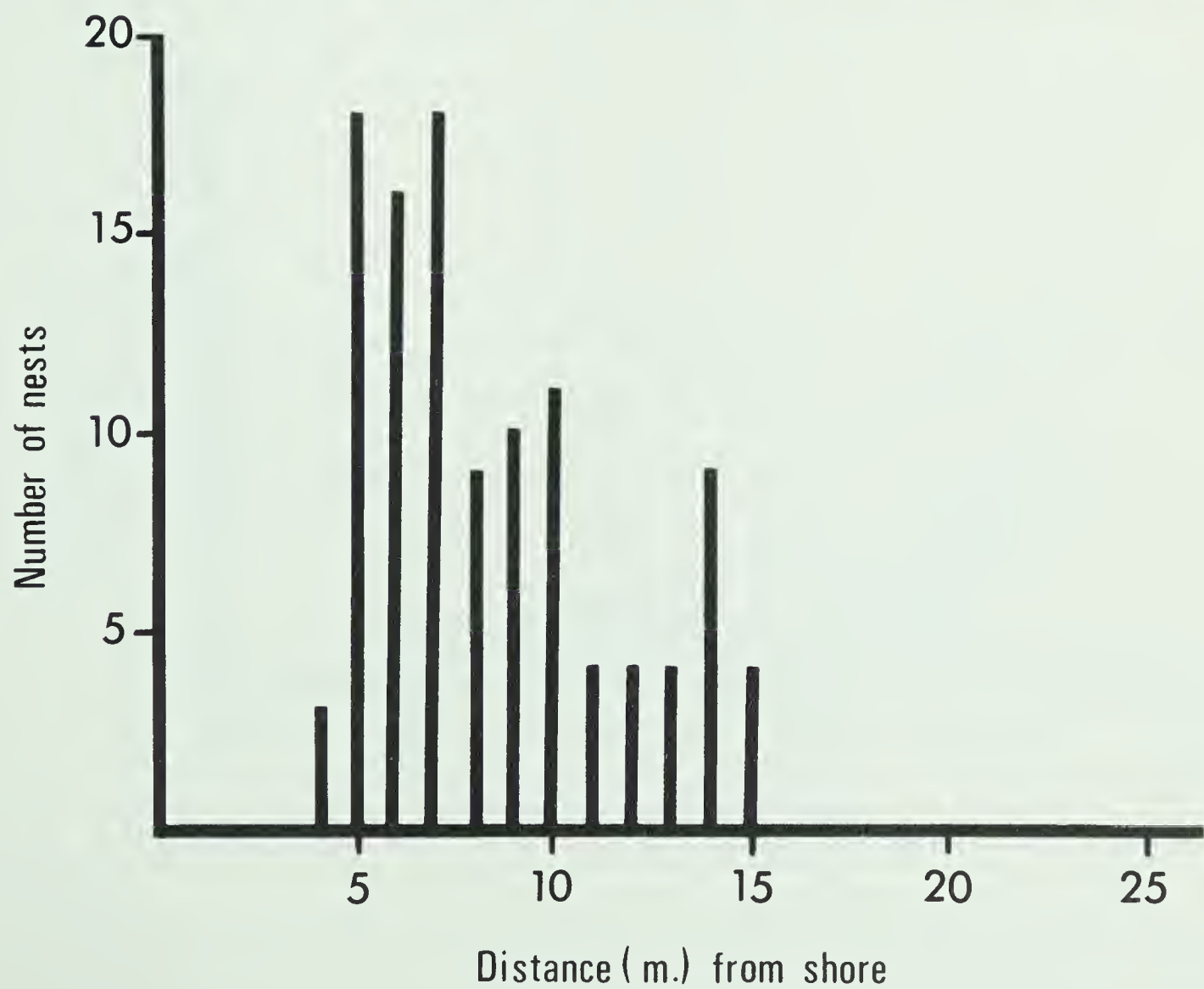
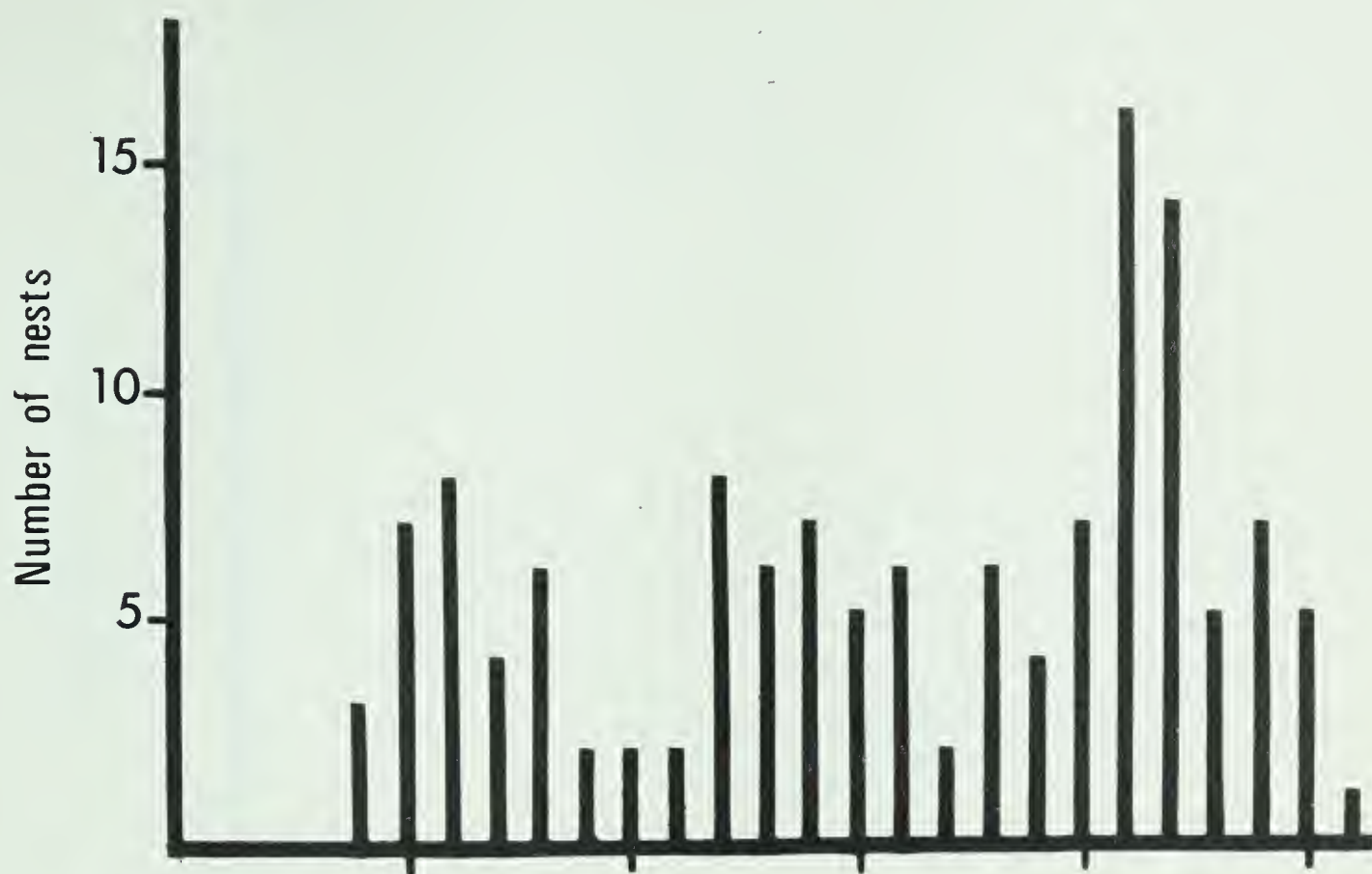


Fig. 12a. Height of stickleback nests above the substrate plotted against the depth of water in which they were constructed. Data obtained from capture of individual males and nests at RESIDENCE POINT. ($n = 79$, $Y = 0.29 + 0.13X$)

Fig. 12b. Height of stickleback nests above the substrate plotted against the depth of water in which they were constructed. Data obtained from transect measurements at RESIDENCE POINT. ($n = 134$, $Y = -12.97 + 0.25X$)

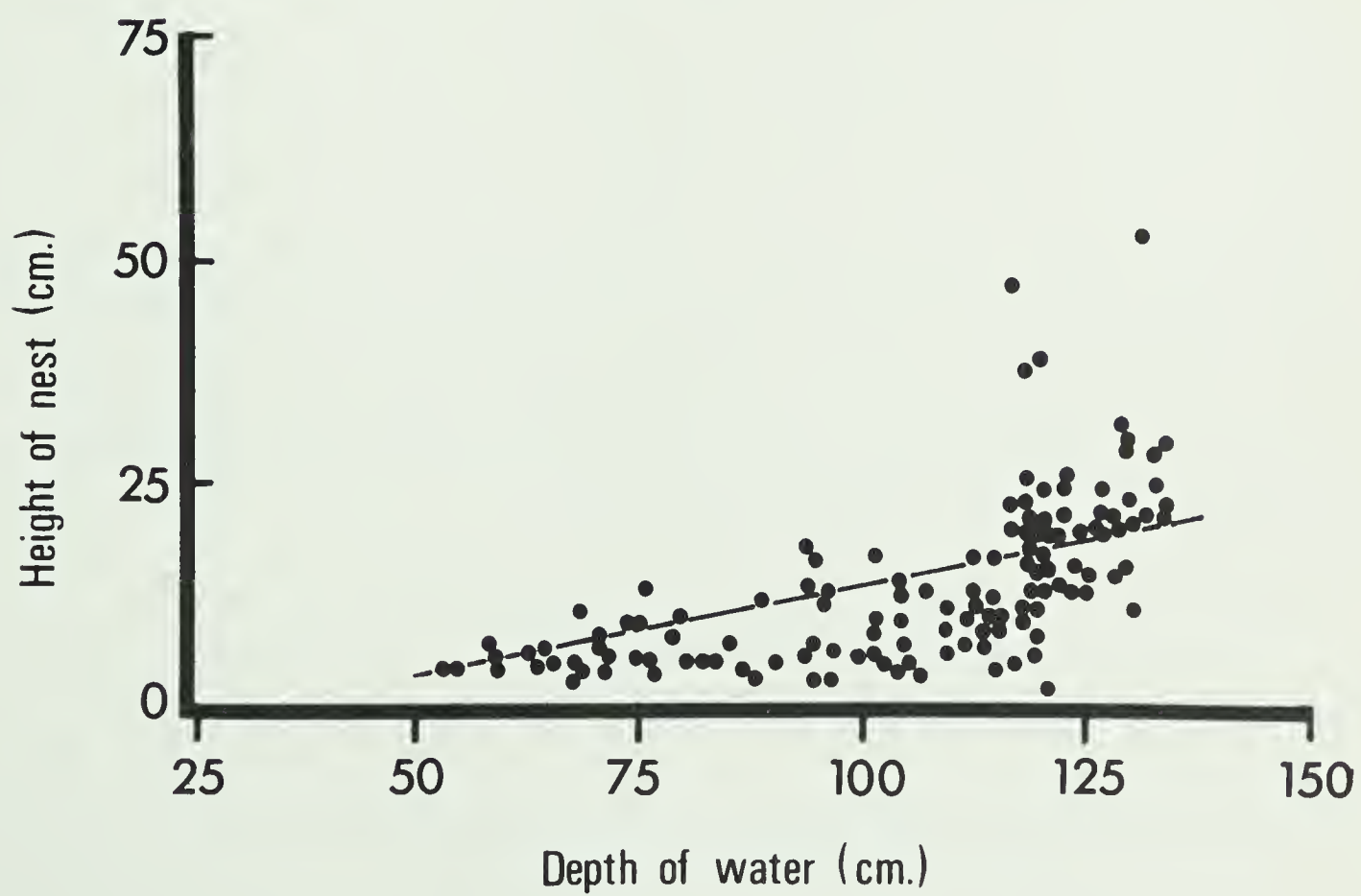
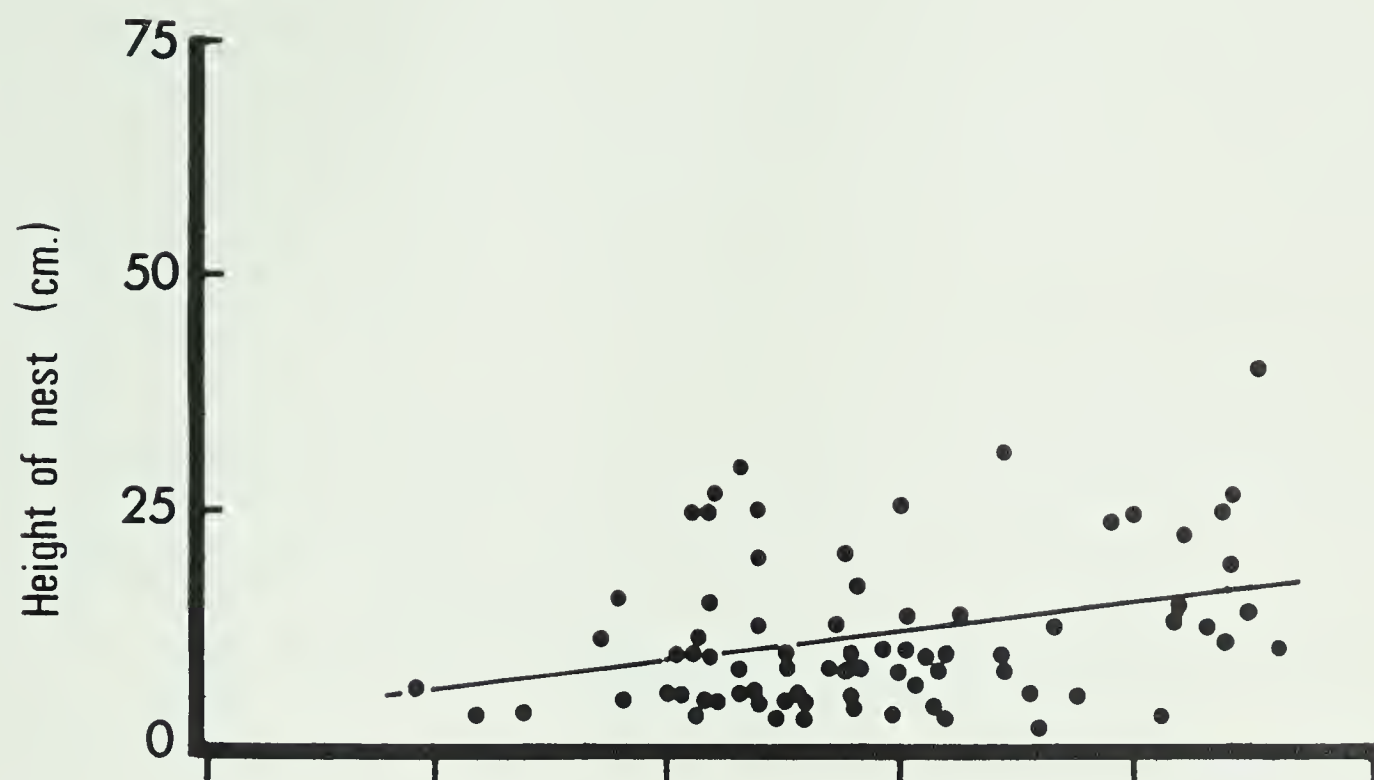
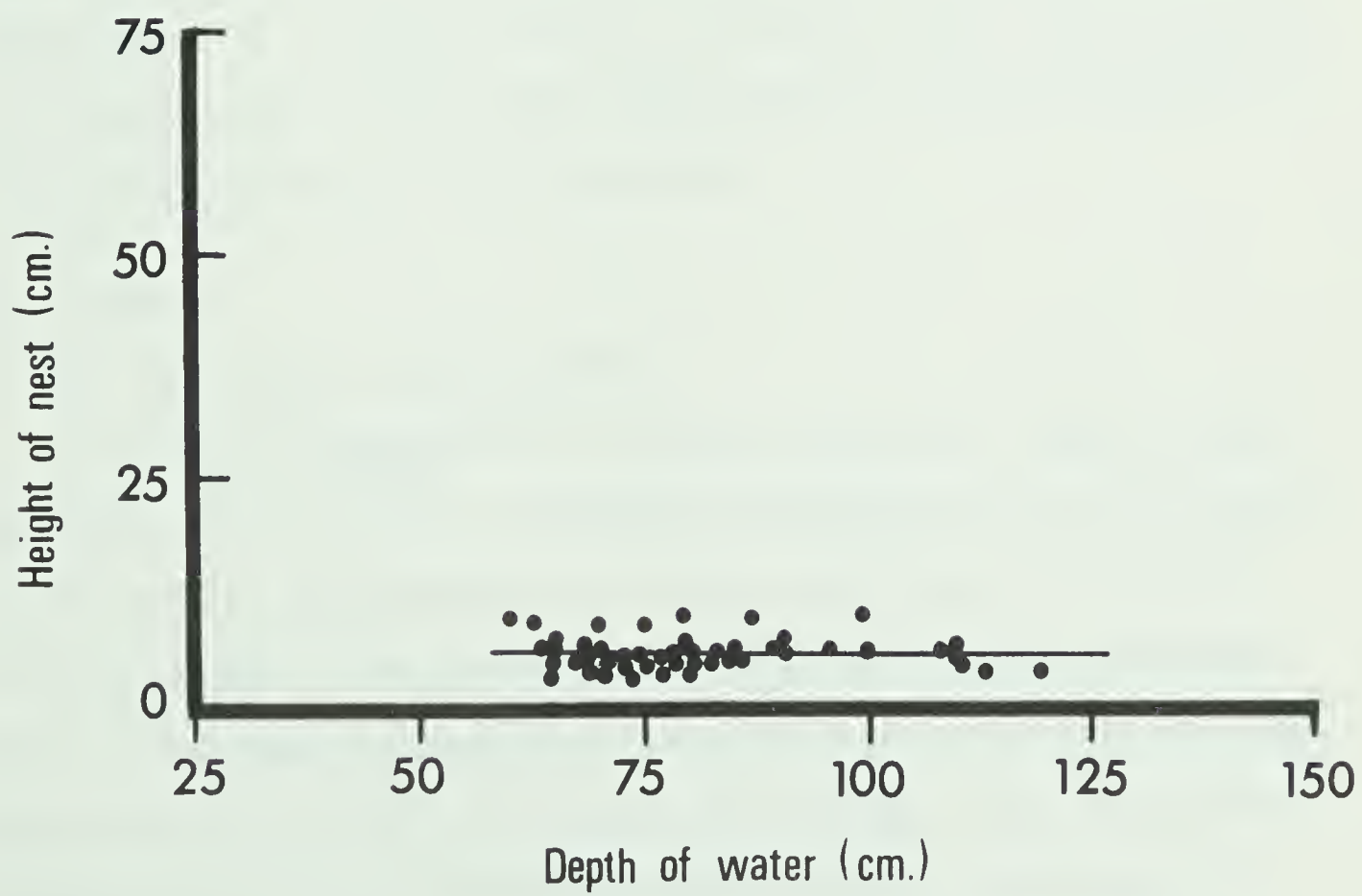


Fig. 13a. Height of nests above the substrate plotted against the depth of water in which they were built. Data obtained from transect measurements at HIGH ISLAND.

$$(n = 90, \quad Y = 6.27 - 0.01X)$$

Fig. 13b. Height of nests above the substrate plotted against the depth of water in which they were built. Data obtained from capture of individual males and nests at HIGH ISLAND.

$$(n = 48, \quad Y = 2.96 + 0.03X)$$



which nests are built above the bottom increases as the water becomes progressively deeper ($r = 0.674$, $p < 0.01$, $df = 125$). Nests in shallow water (75 cm) were located 8 cm above the bottom. In contrast those found in 125 cm of water were about 22 cm above the substrate. Height measurements from individual nest collection also support this correlation ($r = 0.291$, $p < 0.01$, $df = 80$) although greater deviation of values are represented (Fig. 12a). No nests were constructed in waters less than 50 cm deep. The maximum depth of water in which a nest was found was 142 cm at Residence Point.

No direct correlation is detected at High Island between nest height above substrate and water depth ($r = -0.086$, $p > 0.05$, $df = 90$). As the depths ranged from 50 to 125 cm, the nests were usually built 4 to 6 cm above the bottom. Individual nest collection data closely agree with these values ($r = -0.004$, $p > 0.05$, $df = 45$) (Fig. 13b). The shallowest and deepest nests located at this study site were in 59 cm and 126 cm of water respectively (Fig. 13a and 13b).

D. Eggs

1. Description and clutch size

The eggs of Culaea are 1.1 - 1.2 mm in diameter, slightly opaque and light yellow in color. Recently-fertilized eggs within the confines of the nest are united together as a single large mass or in several smaller clumps. I was unable to determine the numbers of females which contributed to a nest clutch except in those nests where the eggs were obviously at different stages of development. The clumping tendency does not persist throughout development. Eyed eggs are loosely scattered throughout the nest algae, possibly to facilitate

better oxygen exchange with the water media.

In four nests where individual clumps were distinguishable from the entire clutch, the egg clumps showed extreme variation in number (38 to 235 eggs). This range of values can be expected since factors of egg mortality, predation and female fecundity are not taken into consideration.

2. Survival

The total egg counts in nests collected are categorized on the basis of embryological development. Some nests contained clutches exhibiting a range of egg development, thus the inclusion of intermediate categories of "uneyed -1/2 eyed eggs" and "eyed eggs -fry".

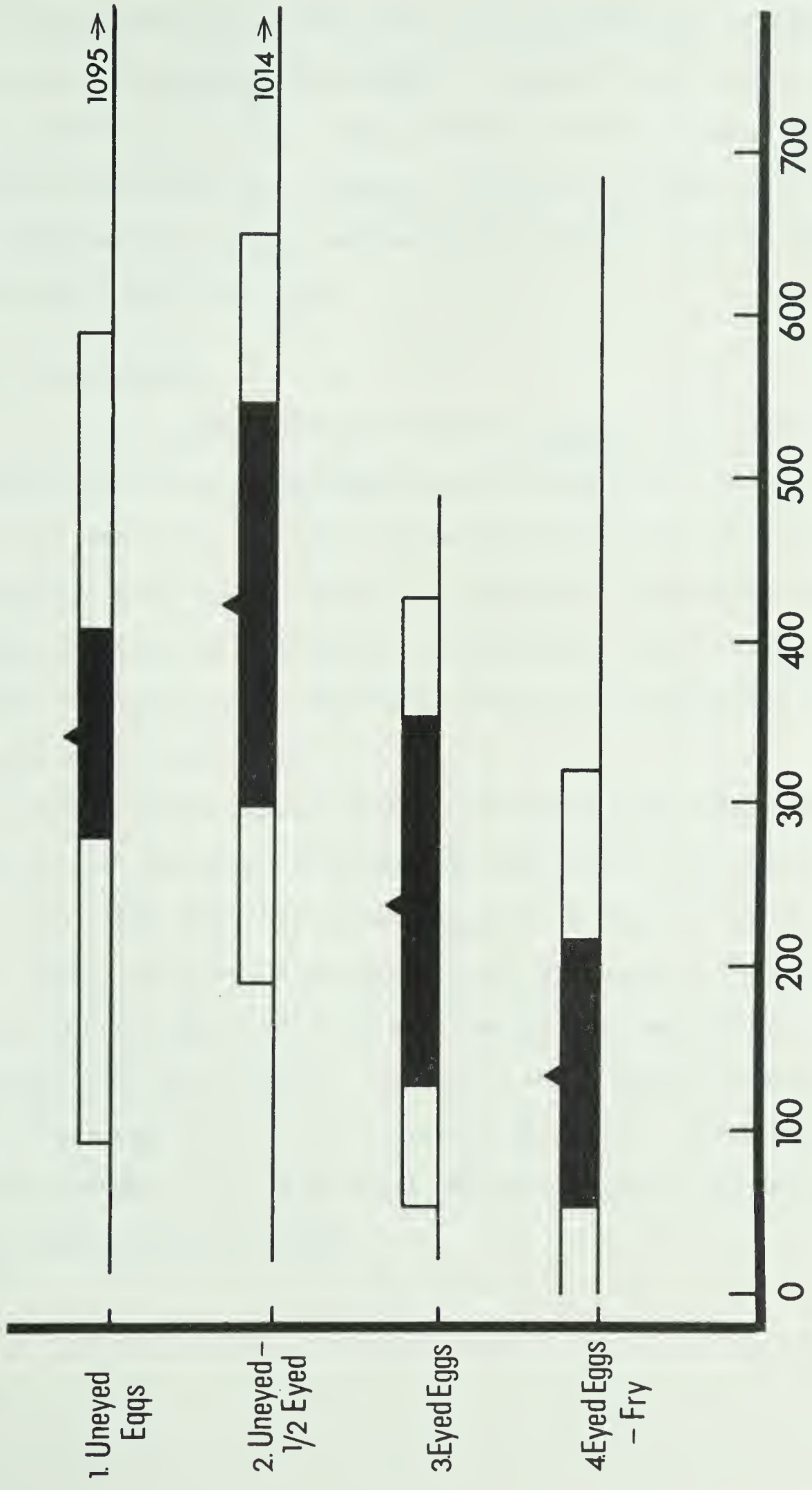
Mean clutch size increased slightly between the "uneyed" stage (342 eggs/nest) and the "uneyed -1/2 eyed" stage (423 eggs/nest). As development proceeded, the clutch sizes diminished to 135 eggs/nest, after which time the fry became free-swimming and left the nest. Although the decrease in clutch size is not statistically significant between successive categories (Fig. 14) with the one exception of categories 2 and 3, the over-all trend is for smaller clutch sizes per nest as egg development continues to hatching. The largest clutch was 1095 eggs, all of which were "uneyed" and tightly aggregated as one mass.

E. Fry Development

Twenty-two of 159 collected nests contained yolked fry ranging in size from 3.1 to 4.6 mm ($\bar{x}=3.8$). In two cases where free-swimming fry were captured along with the guardian male, the fry were approximately 8.0 to 9.0 mm in length. Even at this early stage of growth, the male was having difficulty retaining the active fry as a school.

Fig. 14. Data showing the changes in nest clutch sizes as egg development progressed from fertilization to hatching. Below are listed statistics of unpaired t-test analysis between categories:

1 and 2	$t = 1.21,$	$p > 0.200,$	$df = 80$
2 and 3	$t = 2.22,$	$p < 0.050,$	$df = 24$
3 and 4	$t = 1.54,$	$p > 0.100,$	$df = 30$



Number of Eggs / Nest

During the reproductive period, dipnetting near the surface among the clumps of Potamogeton yielded fry of about 10 to 15 mm in length. These fish were no longer under parental surveillance and protection and were engrossed in searching for minute invertebrate food items.

Hatching time of eggs and incubation periods are not included within the scope of the study.

F. Breeding Males

The mean standard length of 136 male Culaea, individually collected while actively defending nests or territories, was 45.6mm ($s = 3.7$, $s\bar{x} = 0.3$). Sexually mature males in nuptial coloration obtained in seine samples had a size range (31.0 - 65.9mm) which exceeded the size of individually captured males (34.0 - 56.4mm). A larger sample of collected males with nests was necessary to include males of all sizes.

Analysis of the stomach contents of nesting males indicates the frequency of occurrence of stickleback eggs and fry in the diet. The 47 males (35%) which had ingested eggs ($\bar{x} = 12$ eggs, range of 1 - 45) were also guarding nests which contained egg clutches. In addition the stages of development of clutch eggs and the eggs ingested by the guardian male were similar. However 5 males which devoured stickleback eggs did not have a clutch in their nest. Three males had eaten stickleback fry and all 3 fish had fry of similar size under their care at the time of collection.

PATHOLOGY OF INFECTION BY Schistocephalus solidus
PLEROCERCROID ON Culaea inconstans

A. Gross Effects

The presence of plerocercoids is usually detectable in sticklebacks because of the associated distension or ventral pouching of the fish body, particularly in the abdominal region. The ventral and lateral body walls appear almost white in coloration, making the gross swelling of the fish conspicuous even from a dorsal aspect. Under natural and laboratory conditions, infected fish show little or no fear of human activities or disturbance. In fact their curiosity is so keen and their mobility so restricted (also noted by Arme and Owens, 1967), infected Culaea can easily be captured with bare hands as they swim near the surface of the water. Such tameness is absent in non-infected fish.

The body organs particularly the liver and intestinal tract are usually distorted in shape and dislocated by the coiling of the plerocercoid around the viscera. The swim bladder is always intact.

B. Gonad Maturation

The presence of S. solidus plerocercoids invariably cause severe size reduction of host gonads. Although the June 9 sample was collected immediately prior to the onset of breeding, the effects of parasitism were so pronounced that gonads were barely detectable to the unaided eye. Microscopic examination was necessary to determine the sex and stage of reproductive development of infected fish.

1. Females

The ovaries of host females contain only primary oocytes (diameter less than 0.5mm) in the first stage of development. No advanced yolky

eggs were observed. The maximum GS. I. recorded for an infected female was 3.0 (Fig. 15) although values close to the mean of 1.54 ($s = 1.54$, $s\bar{x} = 0.08$) were most common. The mean GS. I. was 8.23 ($s = 5.71$, $s\bar{x} = 0.81$) for non-parasitized females of comparative size.

The incidence of infection tends to fall in larger (older) fish. Most parasitized sticklebacks were between 30 and 35mm in length or about the 1+ year class. The largest of all infected fish was a 43.8mm female (Fig. 15).

The majority (92%) of parasitized fish carried only a single tapeworm larva while two-worm infections made up the remaining 8%.

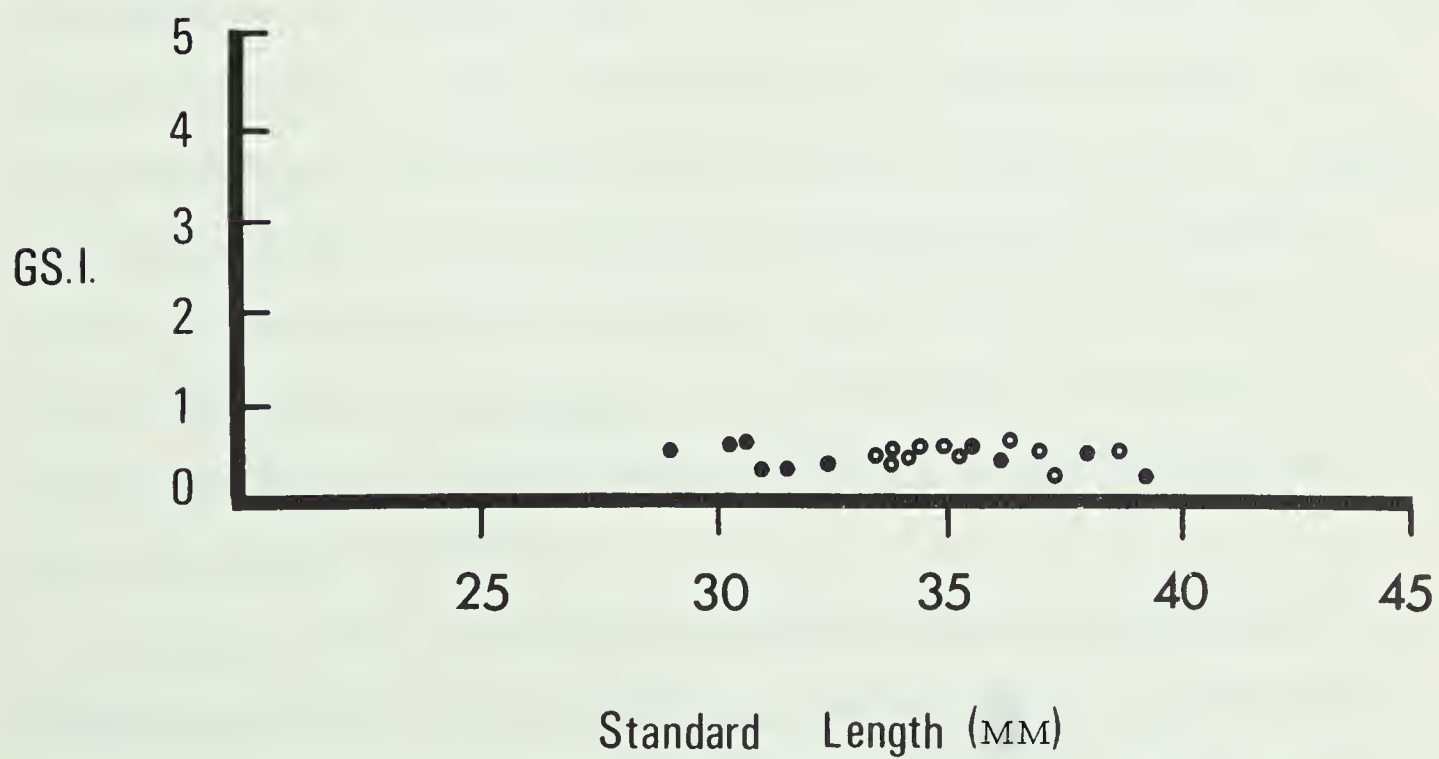
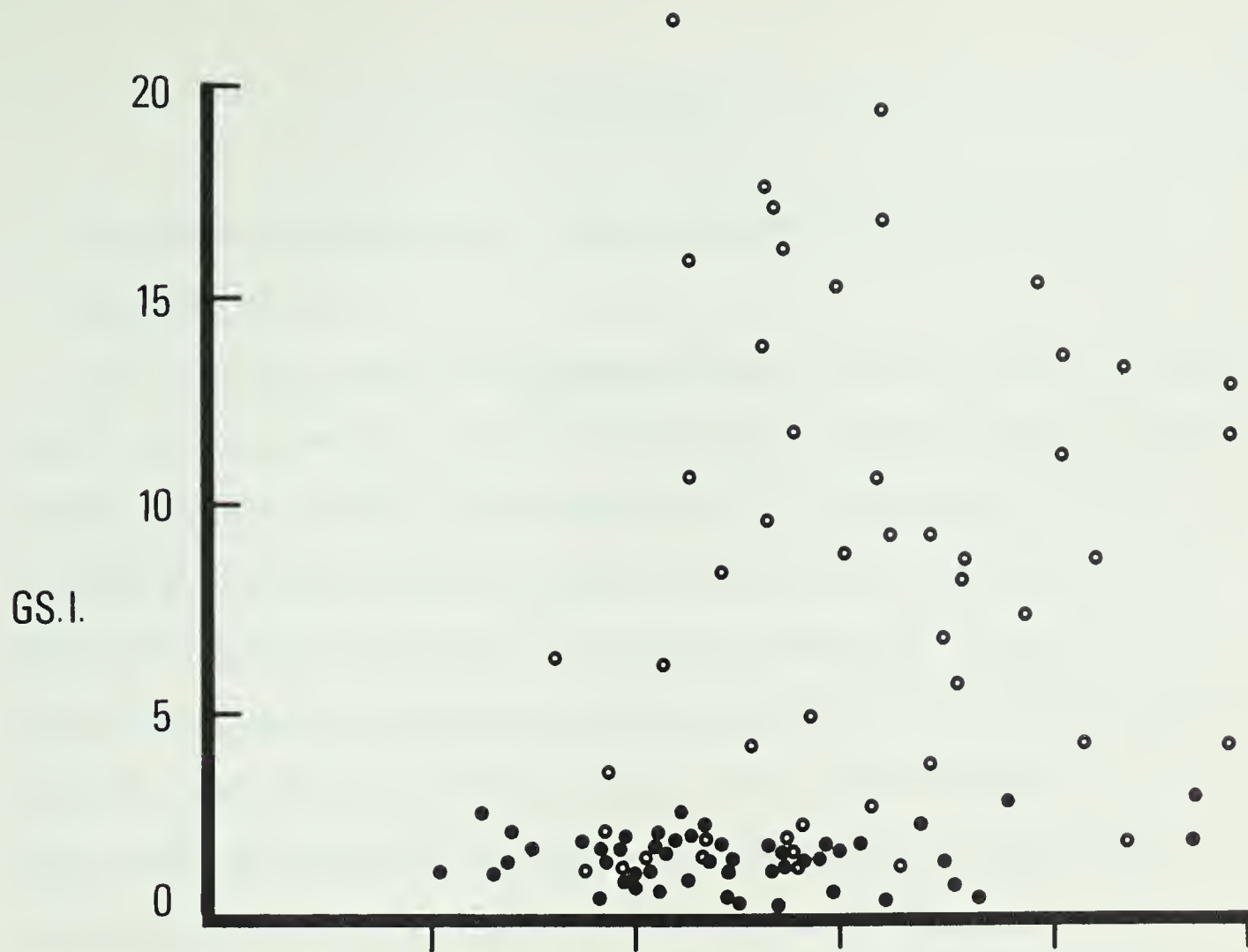
The weight of the plerocercoid, relative to host weight, tends to be inversely related to the size of the fish ($r = -0.51$, $p < 0.01$, $df = 50$). The plerocercoid attained a weight of approximately 0.12 g regardless of the host size and weight. Thus a small fish generally has a relatively greater parasite load than that of a larger individual. The mean parasite load was 19.3% ($s = 5.66$, $s\bar{x} = 0.75$). A 29.9mm female carried the largest plerocercoid load of 31.3%.

2. Males

The data represented in Fig. 16 shows no pronounced differences between the GS. I. values of infected and non-infected males. Because of the minute size of the testes, the use of histological techniques and refined quantitative analysis may demonstrate subtle pathological effects not reflected in the present study. However a parasitized male never exhibits the black nuptial coloration of sexually mature fish. I did not observe an infected individual defending a territory or nest under natural conditions.

Fig. 15. Comparison of ovary development (expressed as GS. I.) of female sticklebacks, non-infected o and infected ● with Schistocephalus plerocercoids, collected just prior to initiation of spawning period, 1970.

Fig. 16. Comparison of testes development (expressed as GS. I.) of male sticklebacks, non-infected o and infected ● with Schistocephalus plerocercoids, collected just prior to initiation of spawning period, 1970.



DISCUSSION

A. Influence of Temperature, Photoperiod and Other Factors on
Reproduction

The breeding season of Culaea generally begins in early spring and ends in early summer. In central New York, Barker (1918) noted that nesting began at 5-10°C and subsided at 21°C during April and May. According to Jacobs (1948), nesting occurred in late March or early April and continued until June in southern Minnesota. The water temperature usually attained a minimum of 8°C before spawning began. Harkness and Ricker (1929) found nests and eggs in Ontario on July 1. Winn (1960) reported nesting males from late April or early May to mid-June in southern Michigan but in northern Michigan spawning was completed about one month later. Temperatures recorded during the nesting period were normally 15 to 19°C, rising in several instances to 27°C. Winn's conclusions are supported by Reisman and Cade (1967) who observed that reproduction is inhibited at temperatures above 19°C in New York ponds. They concluded that the water temperature must not exceed this critical maximum during the breeding season of Culaea. Breeding activities may be influenced by temperature fluctuations in and out of the permissive temperature zone (Reisman and Cade, 1967). Earlier literature listed Culaea as one of the most cold adapted freshwater teleosts with very little resistance to high temperatures (Fry and Fournier, 1942).

In Astotin Lake, the breeding activities of the brook stickleback extended from mid-June to early August during which period the water temperature fluctuated between 16.0 and 26.0°C. The mean monthly

temperature during June and July (peak of breeding) was 21°C , a value well above the critical level of $18.5 - 19.0^{\circ}\text{C}$ determined by Reisman and Cade (1967). In fact the peak nesting activity occurred on July 9 when the water temperatures were reaching daily maxima of 24°C . During the month of May, I recorded temperatures below the "critical level" of 19°C and above the minimum 8°C (Winn, 1960) considered as optimal environmental conditions for reproduction. Neither nesting nor spawning were observed during this same period. The present study suggests that water temperatures of optimal magnitude do not obviously operate, independently at least, as the stimulus of spawning activity.

Several authors reported the integrated action of temperature and photoperiod on the induction of breeding in sticklebacks (Merriman and Schedl, 1941; Van Iersel, 1953; Baggerman, 1957; Harrington, 1959). Reisman and Cade (1967) also suggested that a 14 to 15.5 hour day stimulative photoperiod in coordination with optimal temperatures determined the onset of spawning. Such an optimal photoperiod - 15.4 hours of daylight (recorded at the Province of Alberta Metereological Station, Edmonton) - occurred at Astotin Lake during the first week of May, the same period of optimal temperatures. Nevertheless stickle-back nests were not found until one month later when the average daylength was 17.0 hours.

Photoperiod and water temperature, considered independently or in coordination, do not adequately account for the timing of the reproductive cycle in Astotin Lake. Nesting was deferred one month subsequent to accepted optimal photoperiods and temperatures. When spawning did occur, the fish were subjected to temperatures much above maximum permissive values reported in other studies.

Measurements of oxygen tension, pH, alkalinity and hardness do not suggest that these particular extrinsic factors are integrated cues in the timing mechanism although no "a priori" correlations were anticipated.

B. Reproductive Maturation

Reisman and Cade (1967) postulated the existence of a "short refractory period" during which time the application of optimal environmental conditions seems incapable of stimulating reproduction. Others have shown that physiological consideration of hormone balance in the fish is necessary to understand the timing of fish reproduction (Tromp-Blom, 1959; Hoar, 1962; Ashan and Hoar, 1963).

This study was not physiologically orientated although consideration was given to the anatomical maturation of Culaea. From seasonal examination of female ovaries, I found that only a small minority (less than 14%) of the sampled females were capable of spawning in May. Most fish contained immature ova of less than 1.1 - 1.2 mm diameter and were considered incapable of successful reproduction. Only following a rapid (6 day) increase in the mean GS. I. at the beginning of June were substantial numbers of females sexually prepared for breeding. The onset of spawning 10 days later affirms this reproductive readiness. The absence of any nesting or spawning activities during April and May, months of "optimal" photoperiod and temperature, was thus a function of the sexual immaturity of the female population.

Nevertheless the question is still unanswered: why had not the female Culaea achieved sufficient gonad development to enable them to take advantage of the supposed optimal environmental conditions of late

April, May and early June? Several points of speculation are made. Low spring temperatures associated with the persistent lake ice-cover (until mid-April) may delay the development of reproductive and physiological maturation which occurs during the onset of warm spring temperatures. In Astotin Lake, this seasonal warming of the waters was abrupt (13°C increase in daily means during May). The fish were not adequately prepared physiologically or sexually to take advantage of temperatures and photoperiods within the "optimal" range. This delay in reproductive development forces the stickleback to maximize on the suboptimal (as defined by other workers) environmental conditions.

Obviously the brook stickleback is highly successful in reproduction in Astotin Lake despite temperatures which near its experimentally-determined lethal level and which exceed the accepted permissive range for spawning. Similar broad ecological tolerance is demonstrated by the diversity of habitats utilized by Culaea which include: swampy bogs and lake margins (Trautman, 1957), spring holes (Miller, 1957), sluggish stagnant waters (Barker, 1918), high altitude lakes (Nelson, 1965), and an American artesian well (Bean, 1903). Under such diverse and contrasting circumstances, the same environmental cues, whether temperature, photoperiod, water chemistry, or others yet undefined, may assume different importances to the reproductive biology of Culaea. Although the present study does not quantitatively provide new insights into the timing mechanisms, it does, I hope, show the opportunism and plasticity of the brook stickleback as well as the need for further consideration of natural history of the stickleback throughout its geographic distribution.

C. Nest Ontogeny

Stickleback nests become larger in diameter and over-all size as the egg clutches proceed throughout development. However the actual amount of material incorporated into its fabrication remains relatively the same regardless of the "age" of the nest. Nesting material added by repairing activities (Winn, 1960; Reisman and Cade, 1967) does not contribute significantly to the original mass of materials. Instead the original materials undergo a rearrangement or redistribution to give the characteristic appearance of a fluffy, larger nest structure.

Two possible factors, one passive and one active, may be responsible for this transformation from a compactly woven ball into a tangle of disorganized algae and debris. There is a gradual degeneration of the thread-like secretion employed by the male to mold and hold the materials in place. Without this bonding element, the Rhizoclonium and other nest items are easily dislodged. In addition, male fish were observed under natural conditions and in the laboratory vigorously shaking the entire structure using the head and anterior half of the body inserted through the entrance hole. This activity causes the nest to lose its compact shape and contributes to the nest disintegration. Such behavior occurred only when "1/2-eyed" or "eyed" eggs were present in the nests.

Several advantages accrue from the degeneration of the nest. The male must supply oxygen to the developing eggs by means of fanning water through the nest using his large pectoral fins. As the structure becomes less organized and less dense, the passage of water over the eggs is facilitated. The male can meet the increasing demands for oxygen by the growing embryos to which he devotes considerable

attention (Barker, 1918; Morris, 1958; Winn, 1960; Reisman and Cade, 1967). Once fry have hatched, the degenerated, tangled mass of algae provides an ideal "nursery" for the helpless young. If I accidentally disturbed a nest containing yolked fry while diving, hundreds of fish, temporarily dislodged, would immediately return to and disappear into the ball of algae. If the male had diligently repaired, glued and maintained the nest, the resultant dense bundle would not facilitate such protective behavior. No specially-constructed nurseries were observed in this study (reported by Reisman and Cade, 1967). However, in several cases, a male had relocated his growing and active brood from the nest nursery into the dense mat of Rhizoclonium covering the substrate.

D. Nest Distribution

The literature provides limited data on nest location relative to the substrate or shoreline of the lake. Jacobs (1948) found nests in shallow water of 1 - 3 m. Winn (1960) and Reisman and Cade (1967) observed nests constructed in contact with, to over a foot above, the substrate.

Stickleback nests in Astotin Lake were always constructed within the rooted aquatic vegetation zone of the shallow limnetic waters. Here the tall Potamogeton provided suitable anchorage and support for the nests. This close association explains the limitation and concentration of nests within a 10 m wide and a 26 m wide belt adjacent to the High Island and Residence Point shorelines respectively. Since the rooted aquatics extended to a maximum depth of 135 - 145 cm, the deepest nest was located in 142 cm of water. No nests closer than 4 m (50 - 60 cm depth) from the shore were observed. Frequent exposure to

wind, waves and water level fluctuations would destroy nesting attempts in this zone. Particularly high concentrations of nests found at 5 m from shore (80 cm depth) at High Island and at 20 m from shore (120 cm depth) at Residence Point cannot be explained by the present field data. Further field research supported by laboratory confirmation considering aspects of wind and current exposure, light, plant distribution and predation are necessary to clarify this dispersion phenomenon.

The narrow rooted macrophytic zone at High Island (a function of the moderate substrate gradient) limits nesting to within 10 m from shore. In addition, the prevalence of turbulent, wind-swept waters forces successful nesting close to the bottom regardless of water depth. Wave and wind turbulences are probably reduced near the substrate-water interphase.

In contrast, the calm, protected bay in the lee of Residence Point permits sticklebacks to establish territories and nesting sites at different depths of the water column without threat of damage from inclement weather. However the general trend is to build nests at increasingly greater distances above the bottom in progressively deeper water. Possible causal factors of this direct correlation may include light and predation. The light penetration and daily illumination is greater and longer nearer the surface of turbid, algae-choked waters, as in Astotin Lake. Increased hours of sufficient light for visual observation of possible predators would facilitate successful nest protection and territory defence. In addition, predation by primarily demersal organisms (leeches, Dytiscid larvae, snails) would be minimized because of the relative remoteness of the nest from the bottom. Nests constructed too near or at the surface would be subjected to

wave action and aerial predation of gulls, terns and other piscivorous birds (Kevan, 1970). It was obvious that males which had built nests near the bottom or within the roots of the anchorage plant were concerned about bottom predators. Large depressions were excavated under and around the nest to aid the early detection of possible enemies and robbing male sticklebacks. Early literature reports that such sand-digging is part of the nest-building repertoire of only Gasterosteus (Reisman and Cade, 1967).

E. Egg Survival and Predation

The average number of eggs found in a nest gradually decreases as the clutch development proceeds from fertilization to hatching. The apparent contradictory increment in clutch size between the first two categories is a function of the inclusion of clutches from males which had not finished courting. Once the clutch size is complete, however, various pressures of natural mortality and predation cause a 68% reduction of clutch size up to the time the fry become free-swimming. Stomach analysis reveals that guardian males include stickleback eggs and fry in their diet. The probable source of eggs and young is the nest of the guardian male itself. The evidence supporting this observation is the general similarity in the size and stage of development of ingested and clutch eggs. In only 5 cases (8%) were "guilty" males collected without a nest containing eggs or fry. Predation on the progeny of other males is indicated in these few instances.

Other sources of egg and fry mortality even while under the protection of the attentive parent are the ubiquitous leeches (also noted by Moodie, 1970) and snails of the species Lymnaea stagnalis

and Physa gyrina. During individual collection of nests and males, large leeches would "flush" from the confines of the nest having obviously escaped the attention of the male stickleback. A more common observation was scavenger leeches in abandoned or deserted nests containing diseased eggs or dead fry. In 5% of the collected nests, egg masses of Lymnaea were found deposited among the stickleback eggs within the nest structure. It is not known whether the snail, unobserved by the male fish, deposits the egg mass in the nest or whether the male himself places these eggs among his own progeny. The stickleback may have mistaken Lymnaea eggs for fish eggs of another male which were left unguarded or were abandoned. Experimental designs under laboratory conditions involving the recognition and rejection or acceptance of fish and snail eggs are desirable to describe accurately these data.

F. Retardation of Gonad Growth in Parasitized Culaea inconstans

Infections of Schistocephalus interferes with the development of the genital organs, normal locomotory movements and general behavior of Culaea in Astotin Lake. Data supporting these results are published in reference to Gasterosteidae and Cyprinidae species having infections of cestodes Ligula intestinalis and S. solidus (Arme and Owen, 1967; other workers reviewed by Williams, 1967; Arme, 1968; Arme and Owen, 1968). Severe restriction on normal ovary growth of females caused by the presence of the tapeworm larvae inhibits gametogenesis to such an extent that fully developed ova were never observed. Data analysed from June 9 collections are supported by field observations made during the breeding period. Infected females were not successful contributors to the 1970 spawning population in Astotin Lake.

CONCLUSIONS

1. Sexual maturity of female Culaea inconstans was delayed until late spring, possibly a result of cold water temperatures associated with prolonged spring ice-cover on Astotin Lake. The gonad development of females is rapid once initiated and the population is comprised of 39 - 48% sexually mature females just prior to spawning. However temperature, photoperiod and some aspects of basic water chemistry do not adequately define the mechanisms involved in timing the breeding cycle of Culaea. The onset of spawning failed to coincide with "optimal" environmental standards (8-18°C water temperature, 15 hour photoperiod) reported as critical for successful reproduction. Such data suggests a broad ecological tolerance and a high degree of opportunism for the species.
2. The stickleback nest undergoes an obvious change in appearance and size during egg clutch development. Initially the nest is a small, compact, tightly-bound ball of Rhizoclonium algae but quickly deteriorates into a large, disorganized, undefined clump of decomposing matter. This gradual disintegration, caused partly by natural decay and partly by deliberate activities of the male, may facilitate the passage of water over the oxygen-demanding eggs. The tattered remains of the nest also serve as a protective nursery until the fry are free-swimming. Little or no additional materials are incorporated into the original nest structure by the male during egg development.
3. The distribution and location of nests depends upon the existing environmental conditions such as wind and wave exposure,

fluctuations of lake level, light, predation and plant distribution.

At Residence Point, calm protected waters permit nest construction in the upper sunlit zone of the water column.

Advantages ceded by such placement are better visual detection of enemies and the relative isolation of the nest of eggs from demersal predators. Extremely turbulent water conditions at High Island restrict successful nesting to the bottom regardless of water depth. All nesting activities were observed within the rooted aquatic vegetation zones of predominantly Potamogeton spp.

4. A 68% reduction in clutch size during egg development is a function of cannibalism by guardian males, robbery by marauding fish, predation by leech and snail species, as well as natural mortality from infertility and disease.
5. Female Culaea infected with Schistocephalus solidus exhibit no advanced stages of ova development immediately prior to the nesting period and are incapable of successful reproduction. A parasitized male does not participate in normal reproduction activities such as nest building or territorial defense.
6. The majority of parasitized fish carried only one larva which made up from 9 to 30% of the fish body weight. The larger the fish, the less the proportion of its body weight is attributed to the plerocercoid.

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